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Blood and Bone Marrow Sample Collection and Preparation in Small Mammals

Blood Sample Collection

Blood sample collection is often challenging in small exotic mammals because they can be difficult to restrain, they lack superficial vessels, and the deeper vessels may be covered with fat. As many of these animals are prey species, the process of blood collection will be stressful to the patient. Successful blood collection from small rodents depends in part on successful restraint. Appropriate restraint is accomplished by either manual or chemical means. To reduce the amount of stress on the patient and if restraint is contraindicated, sedation or anesthesia is recommended. In some cases, chemical restraint may be required in order to safely handle the animal (e.g., primates) for blood collection. Injectable, inhaled, and topical approaches, independently or in combination depending on the conditions of the patient, should be considered. The use of chemicals for sedation or anesthesia can alter hematologic parameters, such as packed cell volume, and are discussed in more detail by animal type in Chapter 3. Formal training of the phlebotomist is desirable when obtaining blood samples from some animals in order to prevent the use of frequent or multiple anesthesia and blood sampling. A number of collection sites may be used to obtain blood from small exotic mammals.

Blood Volume Collection

Prior to blood collection, a physical examination should be performed, needed supplies should be gathered in advance, and sample processing and desired diagnostic tests should be determined. Determining and prioritizing diagnostic tests is important to provide a clear understanding of sample processing, given that only a small amount of blood may be obtained. The health status of the animal and its body weight will aid in the determination of the maximum amount of blood that can be safely collected. Often a total blood volume can be estimated given the body weight in kilograms of the animal (Table 1.1). For example, the blood volume of a healthy rabbit is between 5.5% and 7% of the body weight, and up to 10% of the total blood volume may be safely removed at any one time. In general, the maximum amount of blood that can be safely drawn from a patient during a single blood draw is about 10% of the total blood volume or approximately 1% of the total body weight. For example, a blood sample volume of 1.2 mL could be safely withdrawn from a healthy, adult sugar glider weighing 120 g (Johnson-Delaney, 2021). For compromised patients, it is recommended to collect a smaller sample size, such as 0.5% of the total body weight (Smith et al., 2015). The amount of the blood that may pool in a hematoma should also be considered as part of the amount of blood lost from the patient's total blood volume (Mitchell, 2011). The patient's total blood volume will be restored within 24 h of blood collection in most healthy mammals; however, it may take 2 weeks for all of the blood constituents to return to normal. If it is necessary to collect blood from a patient more frequently than every 2 weeks, a smaller sample size (such as 0.5% of the body weight) should be drawn. A maximum of 25% may be removed over a 2-week period (Cooke, 2000). The blood collected from small mammals is typically placed in lithium heparin because the blood sample volume is so small. The heparinized blood sample can then be used for both hematologic studies and clinical chemistries.

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Table 1.1. Blood collection sites and sample volumes for select small mammals.

Species and body weight (BW) range of adults	Collection sites	Blood volume (BV)	Maximum sample volume*
Mice *Mus musculus* (20–63 g)	Lateral saphenous vein Femoral vein Jugular vein Lateral tail vein Ventral tail artery	63–80 mL/kg	7.5–10% BV
Rats *Rattus norvegicus* (225–500 g)	Lateral saphenous vein Femoral vein Jugular vein Lateral tail vein Ventral tail artery Cephalic vein Dorsal tail vein Cranial vena cava	58–70 mL/kg	7.5–10% BV
Gerbils *Meriones unguiculatus* (70–130 g)	Cranial vena cava Lateral saphenous vein Cephalic vein Jugular vein	60–85 mL/kg	7.5–10% BV
Syrian hamsters *Mesocricetus auratus* (87–130 g)	Cranial vena cava Lateral saphenous vein Cephalic vein Jugular vein Gingival vein	65–80 mL/kg	7.5–10% BV
Rabbits *Oryctolagus cuniculus* (varies)	Lateral saphenous vein Cephalic vein Jugular vein Marginal ear vein Central ear artery	5.5–7% BW	7.7 mL/kg
Guinea pigs *Cavia porcellus* (700–1200 g)	Jugular vein Lateral saphenous vein Cephalic vein Gingival vein	70 mL/kg	7–10% BV
Chinchillas *Chinchilla lanigera* (450–900 g)	Jugular vein Lateral saphenous vein Cephalic vein Femoral vein	—	1% BW
Sugar gliders *Petaurus breviceps* (80–160 g)	Jugular vein Cranial vena cava Medial tibial artery Cephalic vein Lateral saphenous vein Femoral vein Ventral coccygeal vein	—	1% BW
Hedgehogs *Atelerix albiventris* (300–600 g)	Jugular vein Femoral vein Lateral saphenous vein Cephalic vein Cranial vena cava	—	1% BW
Ferrets *Mustela putorius furo* (500–2000 g)	Jugular vein Cranial vena cava Lateral saphenous vein Cephalic vein Tail artery (rarely used)	5–6% BW	7.5–10% BV

*From a healthy patient
Sources: Campbell (2015); Quesenberry et al. (2021); NIH (2019).

Restraint and Collection Sites

Myomorphs or True Rodents: Mice (*Mus musculus*), Rats (*Rattus norvegicus*), Gerbils (*Meriones unguiculatus*), Hamsters (*Mesocricetus auratus*)

Blood can be collected from most of the small rodents via the cephalic vein, lateral saphenous vein, lateral tail veins, ventral tail artery (rats), jugular veins, cranial vena cava, femoral vein, and gingival veins. When performing blood collection in small rodents, the patient should be sedated or immobilized, either manually or in some type of a restraint device (a plastic syringe case works well: the patient is placed headfirst into the case) (Machholz et al., 2012; Lindstrom et al., 2015; Frohlich, 2021; Miwa and Mayer, 2021). One hind limb can then be extended by firmly grasping the skin of the leg around the stifle. Simply grasping the skin in this manner often provides sufficient pressure to hold the saphenous vein for venipuncture. If possible, a tourniquet (using a rubber band and hemostat) may be applied to the leg of larger rodents, such as rats. Hair may be shaved from the lateral aspect of the tibia to expose the vein, or the hair may be wetted down with alcohol to facilitate visualization. The vessel is then either punctured with a small-gauge needle and blood is allowed to drip into microcapillary tubes or a microcontainer, or cannulated with a small-gauge needle. Following cannulation, blood is then allowed to drip from the needle hub into a microcollection device. The lateral saphenous vein is typically small and will easily collapse, making the collection of large sample volumes difficult. Similarly, the cephalic vein may be used for blood collection or catheter placement following the same approach used for other domestic mammals, like the dog or cat (Figures 1.1a and 1.1b).

The lateral tail veins can be used to collect blood from gerbils, mice, and rats. The animals need to be sedated or restrained as described above. Consideration must be taken when handling or restraining gerbils to avoid degloving their tails. The veins are located dorsolaterally on either side of the tail and can be dilated by placing the tail under a heat lamp or applying a warm compress prior to blood collection. Occlude the veins by placing a tourniquet at the base of the tail, and use a small-gauge needle attached to a small-sized syringe (e.g., an insulin syringe) to collect the blood sample (Figure 1.2). If the vessels are small, it may be necessary to simply cannulate the vessel with a small-gauge needle, and collect the blood sample in a microcollection device via the drip technique.

The ventral tail artery of rats may be used for blood collection. The rat must be adequately restrained or anesthetized and placed in dorsal recumbency in order to collect blood from this site. The artery is located slightly off the ventral midline of the tail and should be cannulated with a small-gauge needle attached to a 1- or 3-mL syringe with the plunger removed. Insert the needle at a 30° angle at a point approximately one-third the length of the tail from the base of the tail. Once the artery is entered, blood should enter the syringe barrel due to

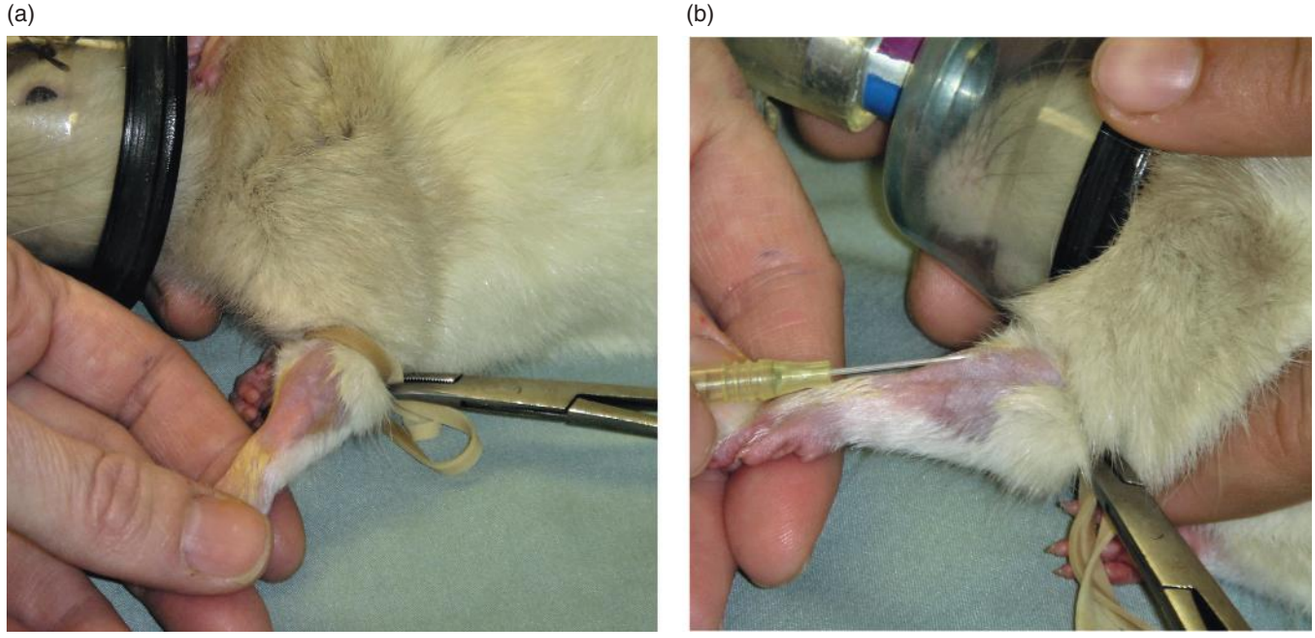


Fig. 1.1. This image shows the approach of using the cephalic vein for blood collection or catheter placement in a rat (*Rattus norvegicus*). (a) The rat is anesthetized, and the region of the forelimb over the cephalic vein is aseptically prepared for catheter placement. A rubber band and hemostat are used as a tourniquet. (b) A 24-gauge catheter is being placed in the cephalic vein of the rat in preparation for surgery.

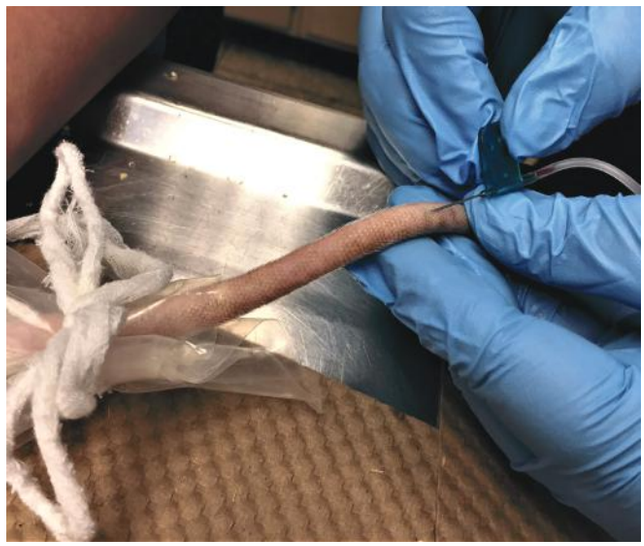


Fig. 1.2. Placement of a butterfly needle into the lateral tail vein for blood collection from a rat (*Rattus norvegicus*).

arterial pressure. After blood collection, apply direct digital pressure to the collection site for hemostasis.

The jugular veins can be used for blood collection and are realistically often the best site for blood collection in small exotic mammals because they are one of the largest accessible veins in a patient. In a laboratory environment, jugular venipuncture is often performed on unanesthetized animals, but in a clinical setting, sedation may be preferable in order to decrease stress on an animal that is often ill and a client's pet. After sedating or anesthetizing the patient, place it in dorsal recumbency and extend the head. The jugular vein is often not visible, but in all rodents lies along a line running from the manubrium toward the ear. The vessel may be occluded digitally at the manubrium, and a small-gauge needle attached to a small-sized syringe (insulin or tuberculin syringe) may be inserted to the hub at a 30° angle.

The cranial vena cava can be used for blood collection in hamsters, gerbils, and rats. This is often a desirable location due to the relatively large vessel size. The animal should be sedated or anesthetized when using this approach. In dorsal recumbency, the animal's forelimbs are held or secured along the sides of their body. The manubrium and first rib are palpated, and a small-gauge needle attached to a small-sized syringe is inserted just cranial to the first rib and within a half of a centimeter lateral to the manubrium (Picazo et al., 2009). The syringe is angled at about 30° from the patient (or table), toward the opposite hind limb, and gently advanced while applying negative pressure with the plunger of the syringe.

The gingival vein has been described for use in blood collection from small rodents in a laboratory setting and, due to the relatively mild traumatic outcome to the tissue and well-being of the animal, is also promising for the clinical setting (Teixeira de Oliveira et al., 2009; Rodrigues et al., 2017). For rodents with short tails or absent tails, such as hamsters and guinea pigs, it provides an additional anatomical location as an option for blood collection. The approach is described in an anesthetized animal in dorsal recumbency. With the lower lip retracted caudally, a 26-gauge needle attached to a 1-mL syringe is inserted just below the gingival-incisor margin along the midline between the two mandibular incisors at a 30–60° angle caudally. The needle is inserted 2–4 mm deep within the mucous membrane of the hamster (Figures 1.3a–1.3e).

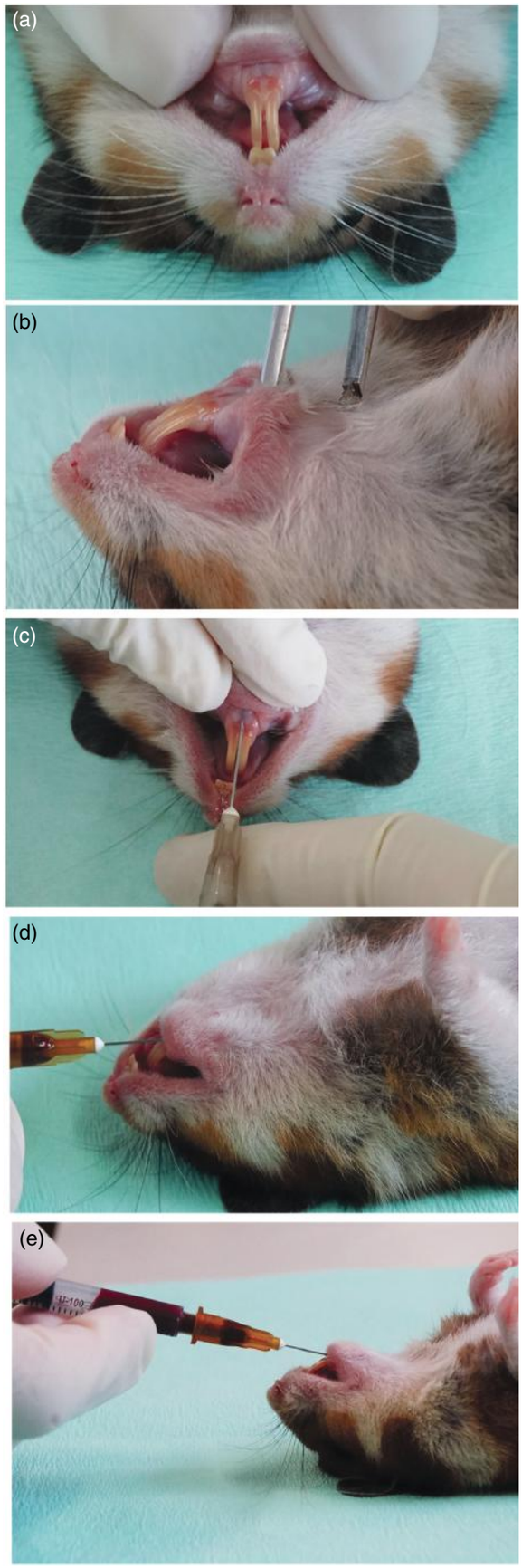
Descriptions of blood collection from the retro-orbital venous plexus/sinus have been described in the literature. This method of blood collection is no longer recommended as a means of sample collection, even in laboratory settings, because trauma to the eye and surrounding structures is very common, and the sample volume that may be collected is very small. Because this procedure is no longer considered a viable means of blood collection, it will not be discussed further in this text.

Rabbit (*Oryctolagus cuniculus*)

The central auricular artery, marginal ear vein, jugular vein, cephalic vein, and lateral saphenous vein are the most used sites for venipuncture in rabbits. Rabbits can be timid patients and in general have a well-developed fight-or-flight response, making the need for adequate restraint an important part of blood collection. Because the rabbit skeleton makes up only approximately 8% of its total body mass, any rabbit that is inadequately restrained is at risk of fracturing its back or long bones. For this reason, the rabbit's hind limbs should never be unsupported or allowed to dangle during restraint. Nervous or intractable rabbits can be restrained using the "bunny burrito." A towel is placed on the exam tabletop, and the rabbit is placed on the towel in approximately the center, with its hindquarters angled into one of the corners of the towel. This corner is folded up over the hindquarters and back of the rabbit. One of the side corners is then folded snugly over the rabbit, followed by the other corner. The remaining corner of the towel can be folded over the rabbit's face. The rabbit can then be scooped up and held in the "football hold" for examination, treatment, or transport.

The lateral saphenous vein is readily accessible, and often a sizable volume of blood can be drawn quickly and easily (Figures 1.4a and 1.4b). Rabbits may be restrained in two ways for access to this vein:

1. The rabbit may be restrained on its side. The handler should scruff the rabbit and restrain it in lateral recumbency, bracing the rabbit's back against the forearm of the hand that is holding onto the scruff. The palm of the other hand should be placed around and over the ventral and caudal aspect of the rabbit's pelvis to stabilize



the hindquarters. The fingers of this hand then wrap around the upper hind limb to hold off the vein. The phlebotomist collecting the blood then extends the rabbit's leg for blood collection.

2. The rabbit is allowed to sit in sternal recumbency on the top of the examination table. The handler restraining the rabbit holds the rabbit's body against her/his own and tucks the rabbit's head into the crook of one arm. The other arm is placed around the rabbit for support. The handler performing the restraint then slides the rabbit toward the edge of the table until the hind leg closest to the edge of the table can drop off. The phlebotomist collecting the sample must kneel on the floor and gently restrain the hind leg in extension to draw the sample. Most rabbits, even intractable ones, will tolerate this method of sampling very well.

The fur over the lateral saphenous vein can be plucked and/or wetted to visualize the vessel. A small-gauge needle and 1- or 3-mL syringe are used to collect the sample. The vessel is superficial under a thin layer of skin, so a steady approach stabilizing the syringe or chemical restraint will help prevent hematoma formation.

The central auricular artery is another commonly used site for blood collection, and most rabbits will readily tolerate blood sampling from this site. The rabbit may be restrained on a tabletop in a "bunny burrito," as described previously, while the skin is prepared by wiping it with an alcohol wipe prior to the venipuncture. Often, application of alcohol will cause vasoconstriction of the auricular artery; however, a topical vasodilation agent such as oil of wintergreen may be applied over the artery, a warm compress applied over the vessel, or subcutaneous acepromazine (0.5–1.0 mg/kg) may also induce vasodilation (Moore et al., 2015). A 25-gauge needle is then introduced into the artery, and the blood is collected into Microtainer® tubes as it drips from the needle hub (Figure 1.5). Collection of blood using this technique is commonly preferred over other methods because the incidence of hematoma formation is relatively low. Some authors, however, do not recommend this method because blood collection from the central auricular artery of rabbits has the potential to cause

Fig. 1.3. Placement of a needle into the gingival vein for blood collection in an anesthetized Syrian hamster (*Mesocricetus auratus*). (a) and (b) Pull the lower lip away from the incisors to expose the vessel located along the midline below the mandibular incisors. (c) A 26-gauge needle on a 1-mL syringe is inserted in the gingival vein. (d) and (e) The lip is released and negative pressure is applied to collect the blood sample. Source: Images courtesy of Marcelo L. Santoro. Rodrigues et al. (2017). Used via license: https://creativecommons.org/licenses/by/4.0/

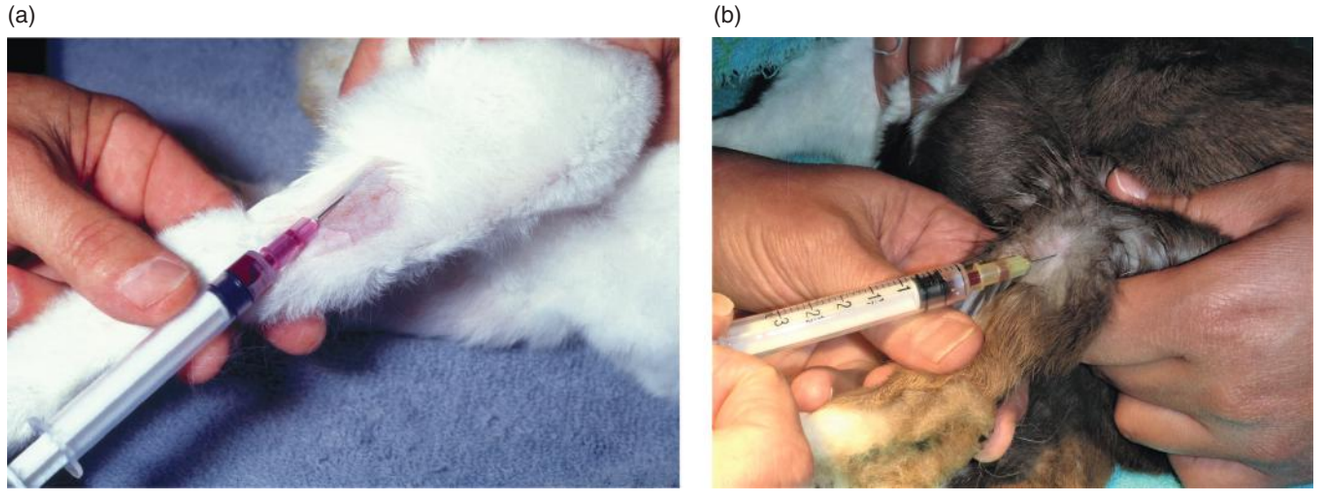


Fig. 1.4. Blood collection from a rabbit (*Oryctolagus cuniculus*) in lateral recumbency using the lateral saphenous vein. (a) The phlebotomist is supporting the syringe with their thumb and middle finger. (b) The phlebotomist is using their thumb as resting support for the syringe.

Fig. 1.5. Placement of a needle into the central auricular artery in preparation for blood collection from a rabbit (*Oryctolagus cuniculus*).

ischemic necrosis of most of the pinna (Malley, 2000). Alternative methods of blood collection from this site include use of a vacuum ear bleeder, or an evacuated glass tube with appropriate needle and needle holder; however, use of aspiration often results in collapse of the vessel in small rabbits. The vacuum ear bleeder method is performed by lacerating an ear vessel and placing the ear inside a flask with a side arm that is attached to a vacuum line and held firmly against the rabbit's head. This method is generally used for research rabbits, where large sample volumes are needed.

Another blood collection site from the ear of a rabbit is the marginal ear vein. Increased blood flow in the marginal ear vein to improve blood collection is made by the application of alcohol, oil of wintergreen, or petroleum jelly over the vein along with finger pressure as a tourniquet. A 23–25-gauge needle is introduced into the vein, and blood is collected directly from the needle hub into collection tubes.

Because some rabbits make a sudden movement as the vein or artery of the ear is punctured, collecting blood into a syringe is facilitated using a butterfly catheter, general anesthesia, or anesthesia of the skin. The skin can be anesthetized using EMLA cream (Astra Zeneca), a mixture of lidocaine and prilocaine that produces a full skin-thickness anesthesia (Flecknell, 2000). The fur overlying the ear vein is plucked or shaved, a 2–3-mm thick layer of EMLA cream is applied, and the ear is covered with a plastic film dressing and a bandage to hold the cream in place. After approximately 45 min, the bandage is removed, and venipuncture is performed. Since the skin is anesthetized, the rabbit does not react to the needle puncture.

Cephalic venipuncture may be performed on the rabbit in much the same manner described for dogs and cats (Figures 1.6a and 1.6b). The handler will restrain the rabbit in sternal recumbency using a "bunny burrito" and present the foreleg while the phlebotomist performs the venipuncture. The fur that overlies the vein is clipped or wetted down with alcohol, and the vein is raised with a tourniquet or held off by the handler providing restraint. A small-gauge needle (25 g) and small syringe (1 cc) are recommended for sample collection because this vein is very small and collapses easily.

Femoral venipuncture can be approached in a manner similar to venipuncture of the lateral saphenous vein. The femoral vein is approached with the rabbit restrained in lateral recumbency and the target leg slightly extended. The medial aspect of the thigh is clipped distally to the stifle, and pressure is applied to the femoral vein in the inguinal canal (Malley, 2000).

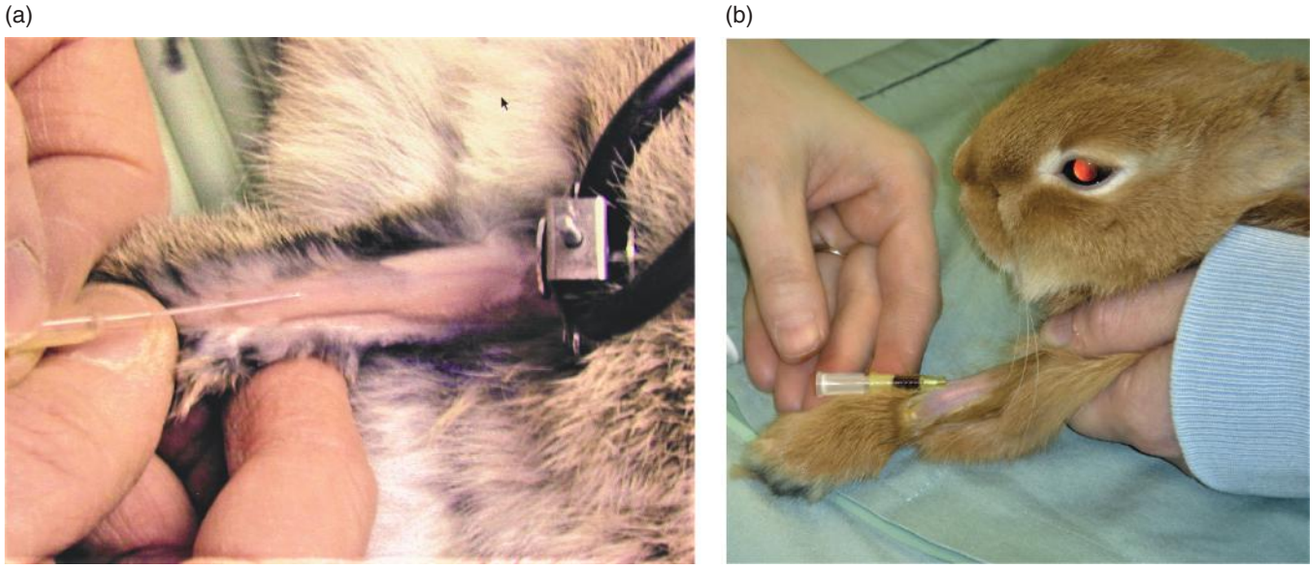


Fig. 1.6. (a) This image shows the cephalic vein of a rabbit (*Oryctolagus cuniculus*), which can be used to obtain blood for hematology studies or (b) the placement of an intravenous catheter.

Rabbits have large paired jugular veins; however, to collect blood from this site, some type of sedation is often necessary because many rabbits do not tolerate restraint and extension of the head. Light to moderate sedation using diazepam or midazolam at 0.5–1.0 mg/kg given intramuscularly 20 min prior to the procedure is recommended (Flecknell, 2000, 2016). Once sedated, the rabbit may be restrained in dorsal recumbency. The front feet are then retracted back alongside the thorax. The phlebotomist performing the venipuncture extends the rabbit's head and tips it back to expose the ventral neck region. Tractable rabbits may be restrained at the edge of a table with the front legs held down and the head extended up (Figure 1.7). If the rabbit struggles, it is best to abort the procedure to avoid back injury. An alternative is to perform jugular venipuncture on a rabbit under a general anesthetic.

Fig. 1.7. Positioning of a chinchilla (*Chinchilla lanigera*) for jugular venipuncture. The animal is held in sternal recumbency at the edge of the table with the forelegs extended down as the head is held in extension.

Guinea Pig (*Cavia porcellus*) and Chinchilla (*Chinchilla lanigera*)

Venipuncture in guinea pigs and chinchillas can be difficult because many of the peripheral vessels are small and difficult to collect blood from. The jugular, lateral saphenous, and cephalic veins are the most used sites for blood collection in these animals. The femoral vein and ventral tail vein may be used for blood collection in the chinchilla as well. The gingival vein may be used in the guinea pig, but is not a reliable site for the chinchilla (Rodrigues et al., 2017; Mans and Donnelly, 2021; Pignon and Mayer, 2021). Warming the patient or applying a warm compress over the vessel may help induce vasodilation to assist with blood collection in guinea pigs (Zimmerman et al., 2015).

Guinea pigs and chinchillas may be restrained for jugular venipuncture in the same manner described for dogs and cats (Figure 1.7). The patient is held in sternal recumbency at the edge of the table with the forelegs extended down as the head is held in extension, and a small-gauge needle (25-gauge) attached to a 1–3-cc syringe is used to collect the sample. The jugular veins of the guinea pig are relatively deep due to the thick neck, and guinea pigs often become stressed during this procedure. It is often best to sedate these animals prior to attempting blood collection from this site. The jugular veins of chinchillas are relatively superficial, and most

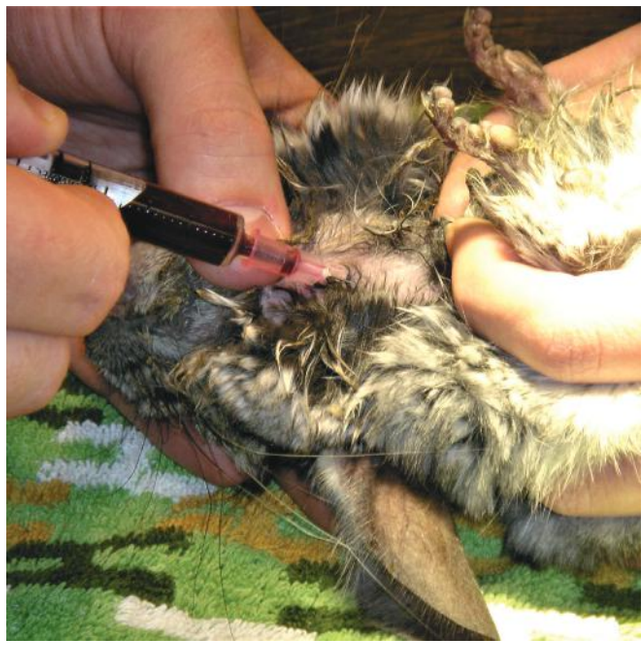


Fig. 1.8. The chinchilla (*Chinchilla lanigera*) is sedated or anesthetized and placed in dorsal recumbency for blood collection from the jugular vein.

Fig. 1.9. A guinea pig (*Cavia porcellus*) restrained by wrapping in a towel (burrito) to aid preparation of blood collection from the lateral saphenous vein.

chinchillas will tolerate restraint for blood collection without sedation, but sedation or anesthesia may be required. In the chinchilla, the vein may also be accessed with the animal in dorsal recumbency (Figure 1.8). The vessels tend to be most accessible if the needle is inserted at a shallow angle just above the sternal notch of the first rib along a line of sight running from the sternal notch to the ear.

The lateral saphenous and cephalic veins are the most accessible veins, but are very small in both the guinea pig and the chinchilla. Both animals may be restrained for cephalic venipuncture in the same manner described for dogs and cats. Use of a guinea pig or chinchilla "burrito" often facilitates restraint during this procedure (Figure 1.9). When performing venipuncture of the lateral saphenous vein, these animals should be restrained in lateral recumbency. Small needles and insulin or tuberculin syringes must be used to prevent collapse of the vein.

The femoral vein may be used for blood collection in the chinchilla. Some form of sedation is recommended when performing venipuncture from this site. The sedated chinchilla may be placed in dorsal or lateral recumbency. Venipuncture is performed in the same manner described for cats using a small-gauge needle and syringe.

The ventral tail vein lies on the ventral midline of the tail and may be used for blood collection in the chinchilla. Anesthesia is recommended when collecting blood from this site. The anesthetized chinchilla is placed in dorsal recumbency and a small-gauge needle attached to a 1–3-cc syringe is inserted at a 30° angle at a point approximately one-third the length of the tail from the base of the tail. After the blood collection, apply direct digital pressure to the collection site for hemostasis.

As described for true rodents, the gingival vein may be used for blood collection in guinea pigs. The sedated or anesthetized guinea pig is placed in dorsal recumbency, and the inferior lip is retracted caudally to approach the vessel. A small-gauge needle attached to a 1-mL syringe is inserted just below the margin of the mucous membrane and mandibular incisors along the midline at a 30–60° angle. Negative pressure is applied as the needle is advanced 3–5 mm into the tissue (Figures 1.10a–1.10e) (Rodrigues et al., 2017).

Sugar Glider (*Petaurus breviceps*)

Blood collection in the sugar glider can be challenging and requires anesthesia. Venipuncture is limited primarily to the jugular veins or cranial vena cava due to the small size of the patient. To perform jugular venipuncture, the anesthetized sugar glider is placed in dorsal recumbency with the forelegs retracted back alongside the thorax. Insert a small-gauge needle (25-gauge or smaller) attached to a small syringe (insulin or tuberculin) along an imaginary line extending from the point of the shoulder toward the mandible. The needle should be inserted at a relatively steep angle along this line and is redirected as needed.

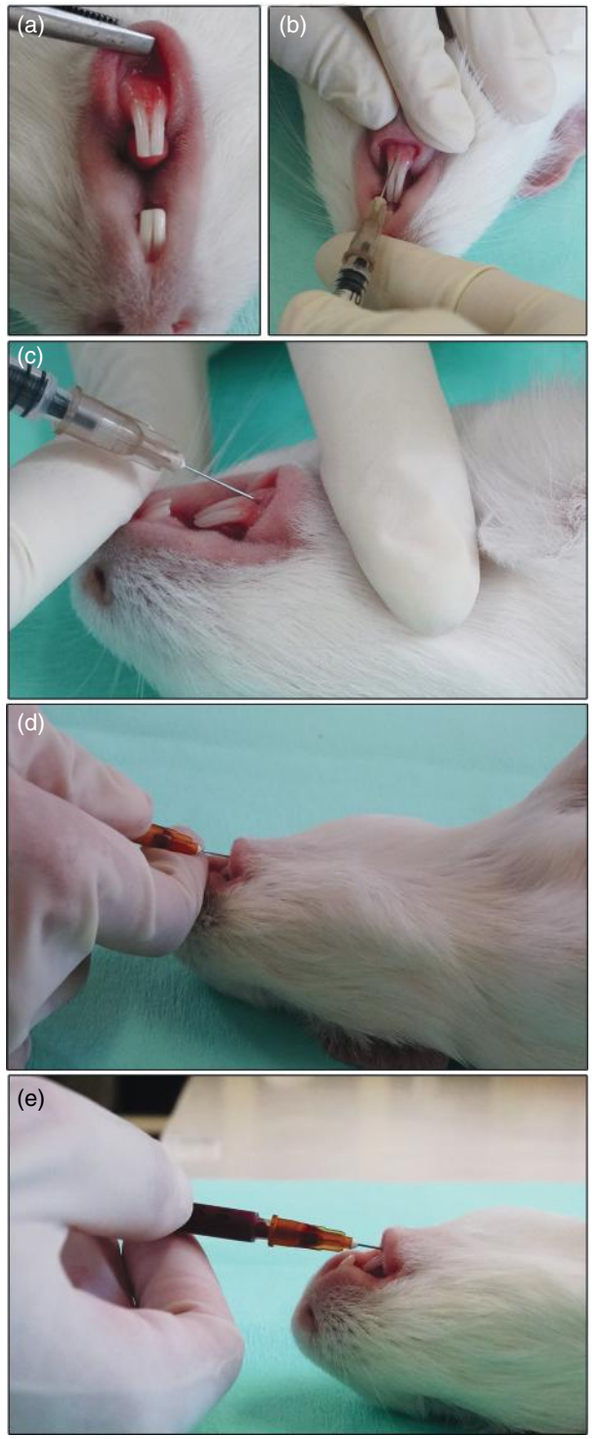


Fig. 1.10. Placement of a needle into the gingival vein for blood collection in an anesthetized guinea pig (*Cavia porcellus*). (a) and (b) Pull the lower lip away from the incisors to expose the vessel located along the midline below the mandibular incisors. (b) and (c) A 26-gauge needle on a 1-mL syringe is inserted in the gingival vein. (d) and (e) The lip is released, and negative pressure is applied to collect the blood sample. Source: Images courtesy of Marcelo L. Santoro. Rodrigues et al. (2017). Used via license: https://creativecommons.org/licenses/by/4.0/

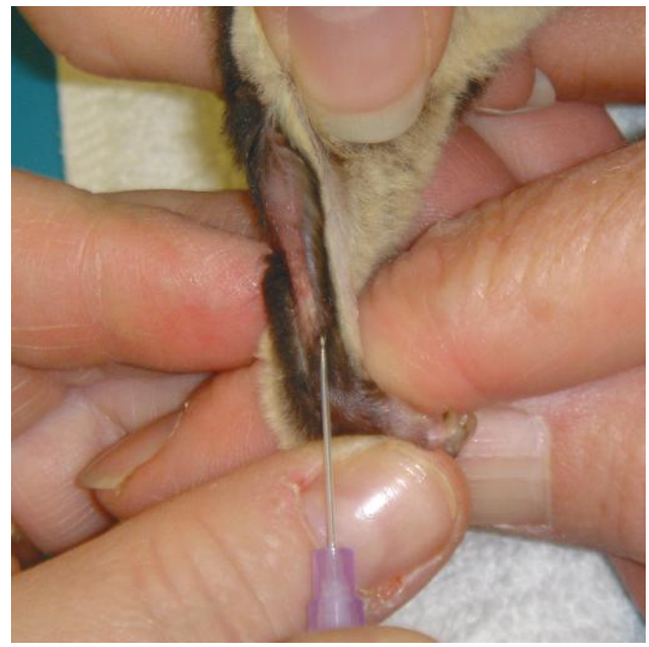


Fig. 1.11. This image shows the cephalic vein of a sugar glider (*Petaurus breviceps*), which can be used to obtain a very small blood sample for hematology or for placement of a 26-gauge intravenous catheter.

The cranial vena cava can be used for blood collection, and the approach is the same as with other small mammals. At the thoracic inlet, using the manubrium and first rib as the landmarks, insert a small needle attached to a small syringe at a 30° angle from the table aiming toward the opposite hind limb.

The peripheral vessels (cephalic vein, lateral saphenous vein, medial tibial artery, femoral vein, or coccygeal vein) may be used to collect very small samples, but may easily collapse (Figure 1.11). A 26-gauge or smaller needle should be used with a tuberculin syringe.

Hedgehog (*Atelerix albiventris*)

Anesthesia is almost always a requirement when performing venipuncture in the hedgehog, because even the tamest hedgehog will usually roll up at the veterinary clinic. Sites available for blood collection include the jugular veins, cranial vena cava, lateral saphenous and cephalic veins, and femoral veins.

Blood is typically collected from the jugular veins because these veins are the largest and most accessible veins present. To perform jugular venipuncture, place the anesthetized hedgehog in dorsal recumbency with the head extended. The jugular vein is rarely visible, but lies along a line of sight extending from the manubrium to the ear (Figure 1.12a). Insert a small-gauge needle (25-gauge or smaller) attached to a small syringe (insulin or tuberculin) along an imaginary line extending

from the point of the shoulder toward the mandible (Figure 1.12b).

The cranial vena cava may be used for blood collection in hedgehogs. The approach is the same as described previously for other small mammals (Figures 1.13a and 1.13b). Caution should be taken with this approach due to the relatively short neck and the cranial position of the heart. Short needles may be used to avoid inadvertent cardiac puncture.

The lateral saphenous, cephalic, and femoral veins may be used for blood collection, but are very small, limiting the amount of blood that may be successfully

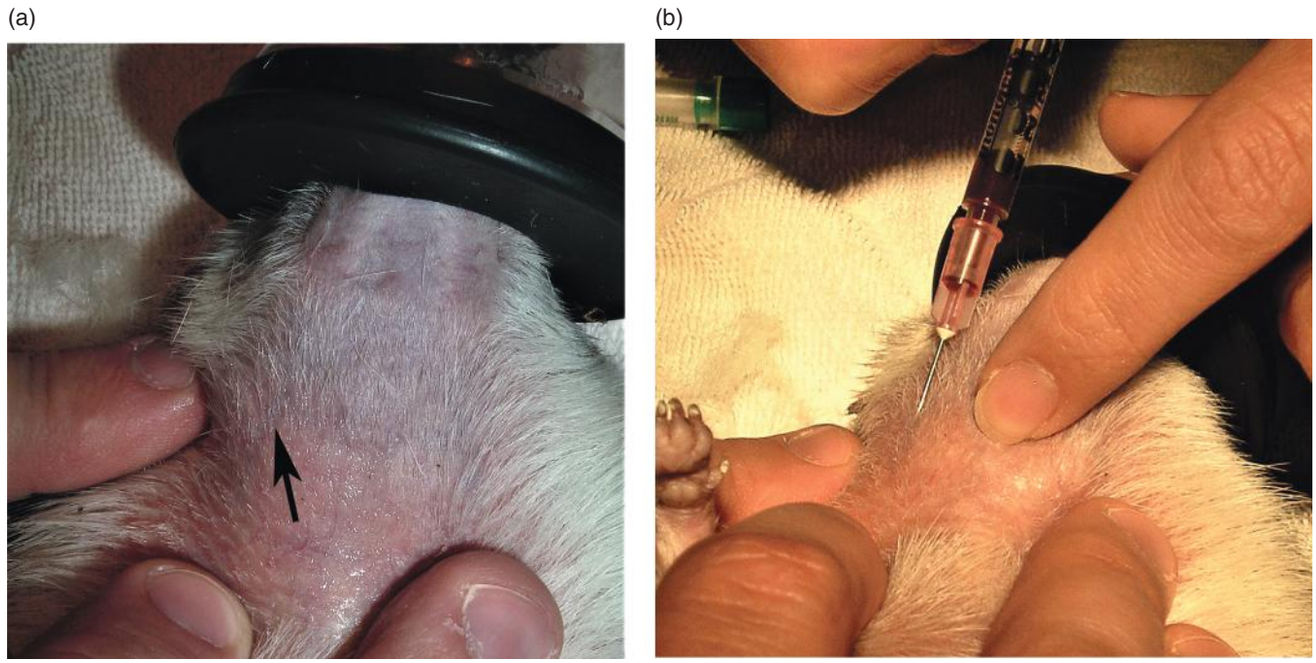


Fig. 1.12. The hedgehog (*Atelerix albiventris*) is anesthetized and placed in dorsal recumbency for blood collection from the jugular vein. (a) The jugular vein (arrow) is visualized in preparation for blood collection. (b) A 25-gauge needle on a 1-mL syringe is used to collect a blood sample.

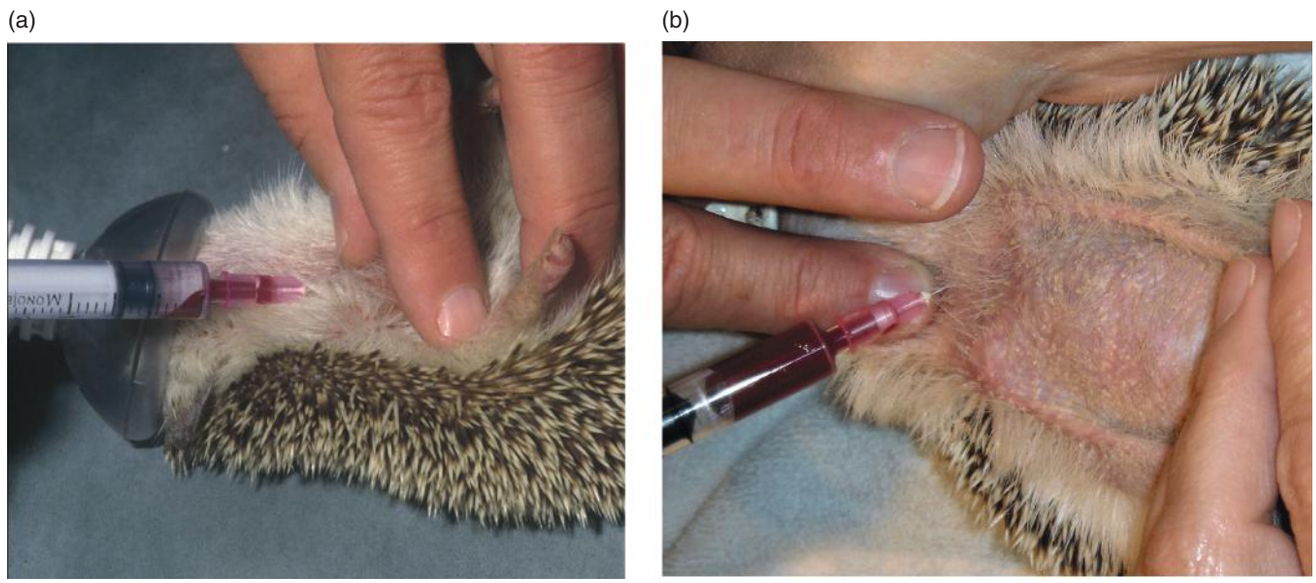


Fig. 1.13. Collection of blood from the cranial vena cava of a hedgehog (*Atelerix albiventris*) by inserting the needle in the "notch" where the first rib meets the manubrium. The jugular vein is also in this area in a shallower position, and a jugular venipuncture is often performed instead when using this method depending upon the depth of the needle insertion. (a) Lateral view, (b) ventral view.

collected from these sites. Blood may be collected using an insulin syringe, or via the drip technique after cannulation of the vessel with a small-gauge needle.

Ferret (*Mustela putorius furo*)

Venipuncture sites commonly used for blood collection in the ferret include the jugular vein, cephalic vein, lateral saphenous vein, tail artery, and cranial vena cava. Clipping a toenail to collect a blood sample should not be performed. It does not yield a viable laboratory sample, and it is painful to the patient.

Most ferrets can be manually restrained for blood collection. Aggressive or intractable ferrets may need to be anesthetized for sample collection; however, it is important to note that anesthesia can alter the hemogram (Marini et al., 1984; Quesenberry and de Matos, 2021). If anesthesia is used for blood collection, this information should be included on the laboratory requisition form.

Blood may be collected from the jugular vein via several techniques. Ferrets may be restrained using the same technique used for cats by holding the forelegs extended over the edge of a table with the neck extending up, or the ferret can be placed in dorsal recumbency and restrained using the same techniques described for the cranial vena cava. A third method of restraint involves wrapping the ferret in a towel with the front legs drawn back along the thorax, leaving only the head and neck extending from the towel. The ferret is then held by the scruff and placed in dorsal recumbency. The jugular veins lie more laterally in the neck compared to dogs and cats and are not always visible, so shaving the neck and applying pressure at the thoracic inlet may enhance the visibility for the phlebotomist (Figure 1.14). Blood samples may be collected with a 22- or 25-gauge needle on a 1–3-cc syringe. Ferrets that struggle or are excessively resistant to manual restraint should be anesthetized.

The cranial vena cava may be used for blood collection from ferrets; however, a jugular venipuncture is often performed when attempting to obtain blood from the anterior vena cava. The jugular vein is frequently encountered just under the skin in the same location as the approach to the cranial vena cava. Those unfamiliar with ferrets or having minimal experience in phlebotomy should perform this procedure on anesthetized ferrets until they become proficient with this technique, to avoid laceration of the vena cava and harm to the ferret. Restraint is very important and often requires two to three people when performing this method of blood collection on unanesthetized ferrets. One handler will restrain the forelimbs and head of the ferret while a second handler restrains the hind limbs and body. A phlebotomist draws the blood sample by palpating the "notch" where the first rib meets the manubrium and visualizing a line of sight running caudally from the "notch" across the midline of the ferret's body to the opposite hind leg. A 25-gauge needle attached to a 1–3-cc syringe is inserted at a 45° angle into the thoracic cavity (for a cranial vena cava blood collection) or just under the skin (for a jugular venipuncture) at the notch and aimed along the sight line toward the opposite hind limb (Figure 1.15). Negative pressure is applied to the syringe, and the needle is slowly withdrawn until blood begins to fill the syringe; at which point the needle is positioned in the vessel and should remain steady as the syringe fills with blood to the desired safe amount. If the ferret struggles, the syringe should be withdrawn

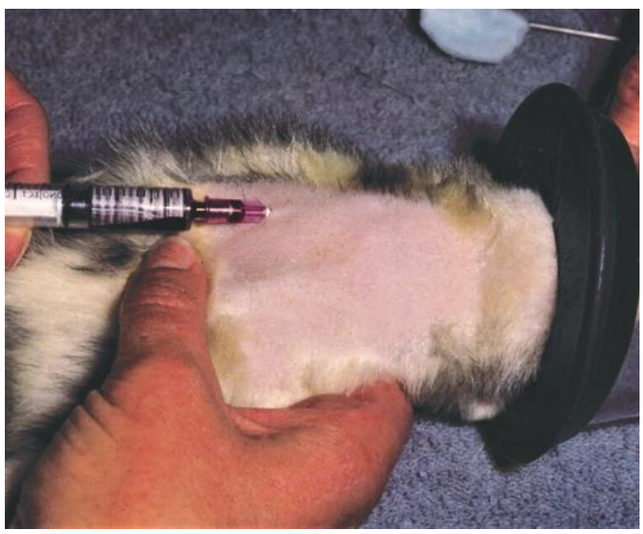


Fig. 1.14. Blood collection from a ferret (*Mustela putorius furo*) using the jugular venipuncture method.

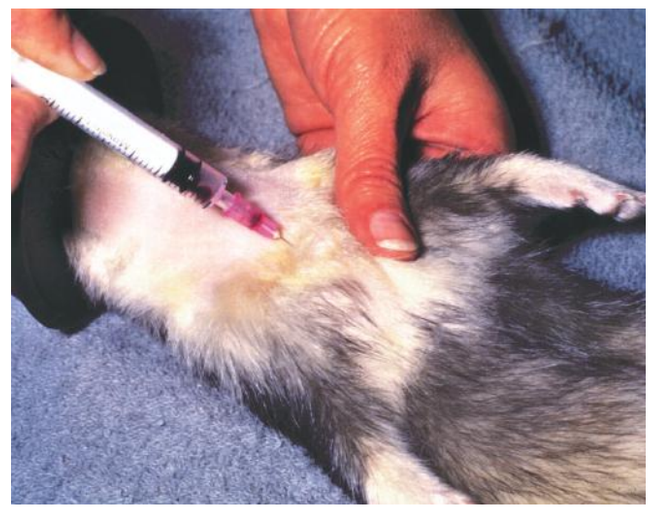


Fig. 1.15. Collection of blood from a ferret (*Mustela putorius furo*) using the cranial vena cava approach by inserting the needle in the "notch" where the first rib meets the manubrium. The jugular vein is also in this area in a shallower position, and a jugular venipuncture is often performed instead when using this method depending upon the depth of the needle insertion.

Fig. 1.16. Collection of a blood sample from the cephalic vein of a ferret (*Mustela putorius furo*).

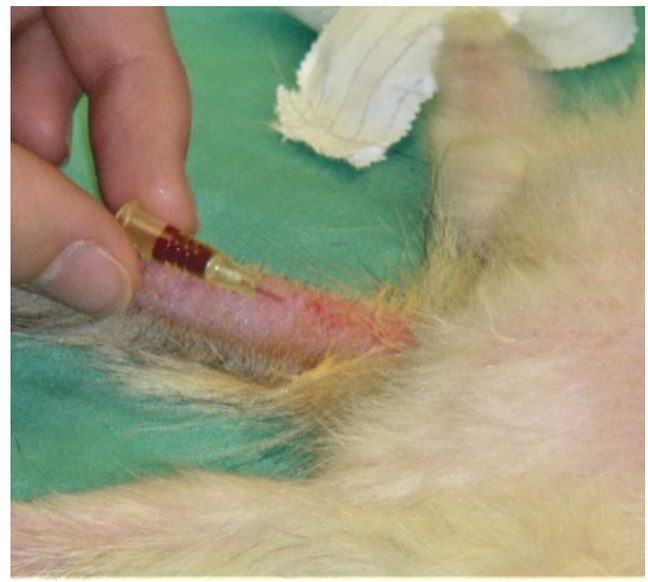


Fig. 1.17. A 24-gauge catheter placement in the ventral tail artery of a ferret (*Mustela putorius furo*).

quickly. A second attempt should not be made until the ferret is quiet. Any ferret that struggles excessively during this procedure should be sedated before attempting blood collection from this site.

The cephalic vein is located over the antebrachium, courses laterally, and may be used for the collection of small volumes of blood (up to 1 mL) using a 25-gauge needle attached to a 1-mL syringe. Ferrets may be restrained for cephalic venipuncture by wrapping them in a towel with the head and one foreleg free. The head should be restrained by extending it upward, while the foreleg is presented in extension (Figure 1.16). The vein may be occluded by simply placing the thumb or a finger over the dorsal surface of the elbow by the handler who is restraining the ferret. The cephalic vessel is small and easily collapses, so it is not necessary to roll this vessel as is commonly done with dogs and cats. If additional stability is required, the handler can place his or her forearms on the tabletop on each side of the ferret to support the body. Most ferrets will struggle briefly, and then will become quiet. A 1-cc syringe with a 25-gauge needle is used to collect the blood sample.

The lateral saphenous vein courses diagonally across the lateral surface of the hind leg just proximal to the hock and may be used for collecting small volumes of blood. A ferret may be restrained for blood collection from this site by scruffing it with one hand and laying it on its side. The handler then places the palm of the other hand over the sacrum and pelvis for support and holds off the vein by wrapping his or her fingers around the leg above the stifle. Occasionally, a second handler is needed to help restrain the ferret's body. A 25-gauge needle mounted on a 1-cc syringe is used for sample collection.

The tail artery may be used for venipuncture when necessary, but is not a common or preferred site for blood collection in the clinical setting. This site may be more painful for the ferret than other blood collection sites. The ferret may be restrained by either scruffing and restraining on its back, or by anesthetizing it and placing it in dorsal recumbency. A 25-gauge needle attached to a 1-mL syringe is then inserted at a 45° angle toward the body on the ventral midline of the proximal third of the tail and advanced until blood begins to fill the syringe (Figure 1.17). Direct digital pressure must be applied for at least 3 min after blood collection to provide adequate hemostasis.

Other Collection Sites and Considerations

The cranial vena cava has been used to collect blood from ferrets, small rodents, hedgehogs, sugar gliders, and other small exotic mammals, and many clinicians use this approach as a first choice of blood collection (Figures 1.13 and 1.15). This method of blood collection is very "safe"; however, there is high potential for complications such as intrathoracic bleeding, bleeding into the pericardial sac or mediastinum, or inadvertent cardiac puncture in small patients. The phlebotomist performing blood collection using the cranial vena cava must be aware of the thoracic anatomy of the animal they are intending to perform venipuncture on and must consider the size and length of the needle being used.

The femoral vein and artery are other considerations for blood collection in several mammals, such as rodents, sugar gliders, and other small mammalian species due to the potential of a relatively moderate volume collection. The animal may need to be sedated or placed under general anesthesia. In dorsal recumbency, the vessels are located on the medial aspect and proximal third of the

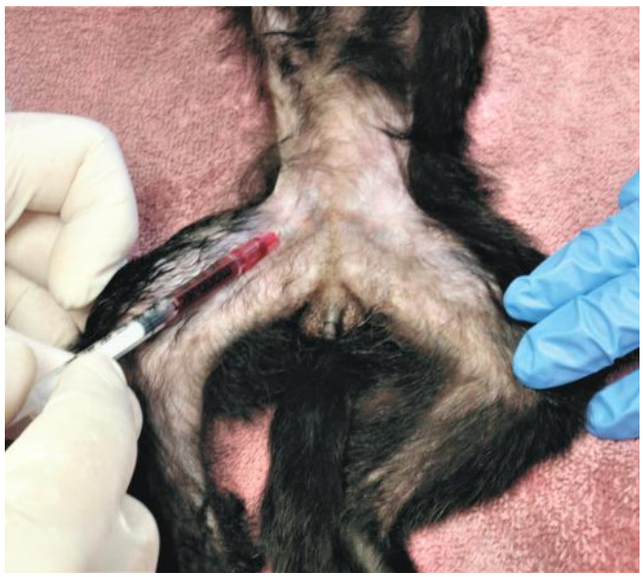


Fig. 1.18. Collection of a blood sample from the femoral vessel of a Goeldi's monkey (*Callimico goeldii*).

thigh. A small-gauge needle attached to a 1- or 3-mL syringe is inserted parallel to the femur just distal from the junction of the limb and body (Figure 1.18).

Cardiocentesis can be used to collect blood from small mammals, but is typically only performed for terminal procedures because of the risk of causing death during the procedure. Cardiocentesis must be performed under general anesthesia to ensure that there is no movement of the patient, and because the heart may move away from the needle during the procedure. To perform cardiocentesis, place the anesthetized animal in dorsal recumbency and locate the heart by palpation. Insert a needle attached to a syringe slightly to the left of the manubrium and advance it toward the heart.

Blood Sample Handling and Preparation

Blood for hematology is typically collected into tubes containing an anticoagulant, such as ethylenediaminetetraacetic acid (EDTA) or heparin. Blood collected for clinical biochemistry analysis is collected into tubes containing heparin or no anticoagulant. The blood sample from a small mammal is prepared for hematology studies in the same manner as described for domestic mammals (Weiser, 2012, 2022). Likewise, the hematologic methods used to evaluate the hemic cells in blood samples from these animals are the same as described for domestic mammals.

Bone Marrow Collection

Cytologic evaluation of a bone marrow aspiration biopsy is often performed as a diagnostic aid when an unexplained hematologic abnormality such as nonregenerative anemia, neutropenia (heteropenia), thrombocytopenia, myeloproliferative or lymphoproliferative disorder (leukemia), gammopathy, or suspicion for neoplasia is present. Bone marrow biopsy needles or spinal needles are required for this procedure because they have a stylet to facilitate passage of the aspiration needle through the cortical bone into the marrow space. The type of biopsy needle used for aspiration depends upon the size of the animal, location of the biopsy site, and preference of the cytologist. Bone marrow biopsy needles commonly used for marrow collection in domestic mammals and humans can also be used for marrow collection in birds and reptiles (Jamshidi bone marrow biopsy/aspiration needles and disposable Jamshidi Illinois sternal/iliac aspiration needles, Kormed Corp., Minneapolis, MN) (Figure 1.19). The pediatric sizes are preferred because of the relatively small bone size of most small mammals, birds, and reptiles compared to the larger domestic mammals. Spinal needles containing a stylet can be used for marrow collection in very small animals as well.

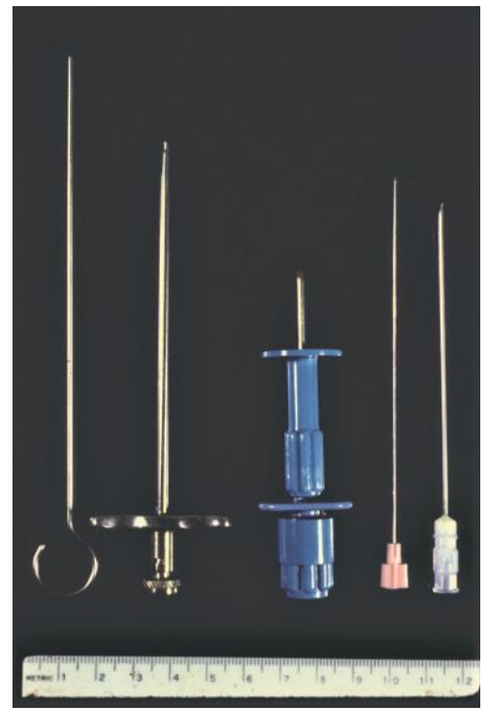


Fig. 1.19. Representative types of aspiration or core biopsy needles used to obtain bone marrow samples, including (left to right) pediatric Jamshidi bone marrow biopsy/aspiration needle (Kormed Corp., Minneapolis, MN), disposable Jamshidi Illinois sternal/iliac aspiration needle (Kormed Corp.), and disposable spinal needle.

The most common site for bone marrow aspiration biopsy or core biopsy in small exotic mammals is the proximal femur (Figures 1.20–1.25). Additional sites could include the proximal tibia, proximal humerus, and the ileum; however, the small body size of many small

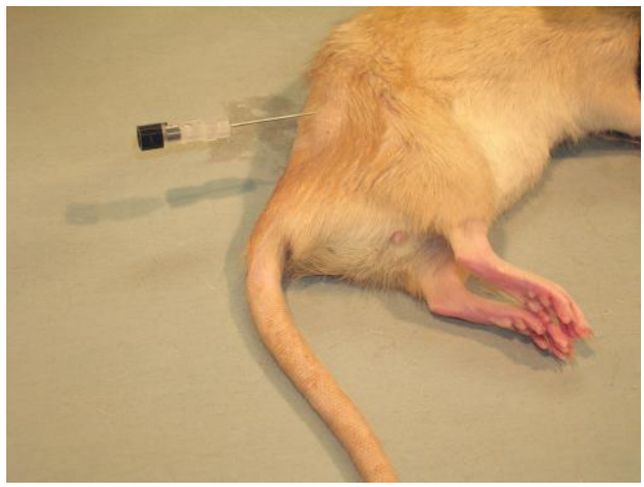


Fig. 1.20. Placement of a spinal needle into the proximal femur of a rat (*Rattus norvegicus*) for a bone marrow aspiration biopsy.

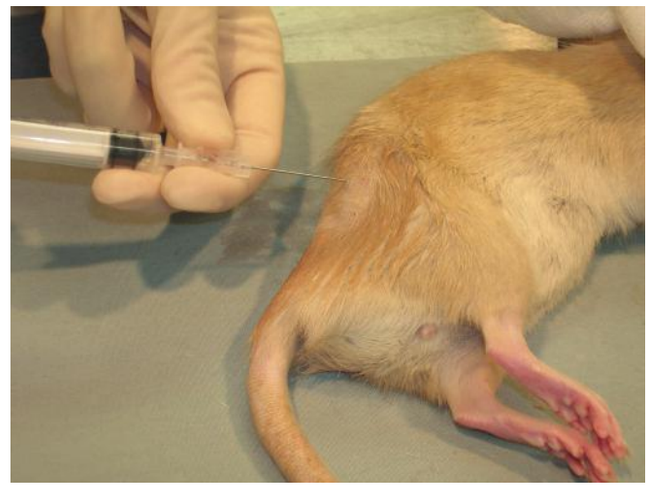


Fig. 1.22. Aspiration of the marrow sample into the lumen of the spinal needle that was placed into the proximal femur of a rat (*Rattus norvegicus*) for a bone marrow aspiration biopsy.

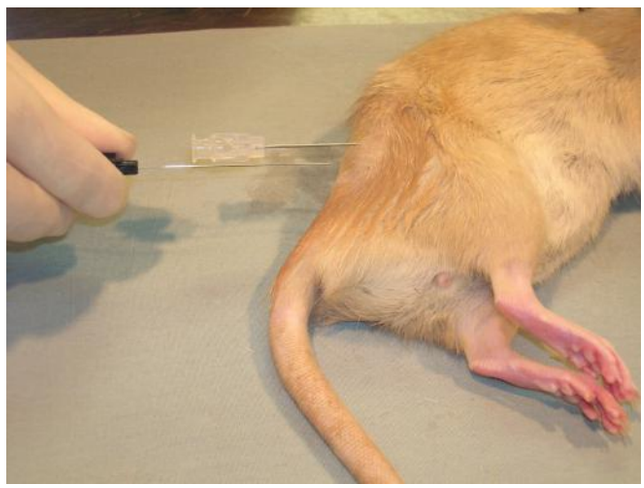


Fig. 1.21. Removal of the stylet from the spinal needle that was placed into the proximal femur of a rat (*Rattus norvegicus*) for a bone marrow aspiration biopsy.

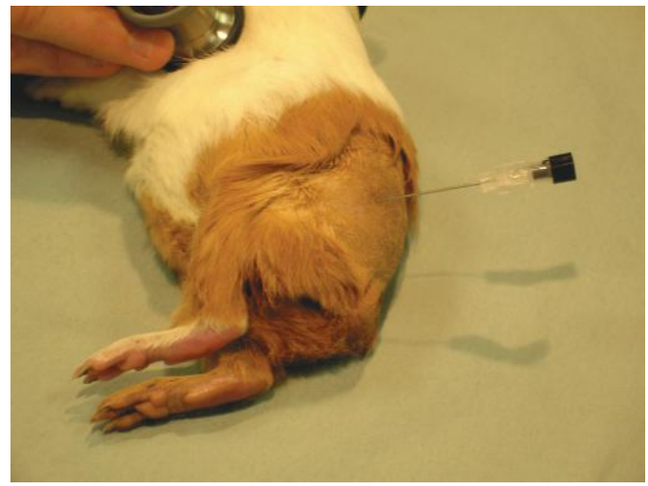


Fig. 1.23. Placement of a spinal needle into the proximal femur of a guinea pig (*Cavia porcellus*) for a bone marrow aspiration biopsy.

exotic mammals precludes the use of these sites for bone marrow collection.

When performing a bone marrow aspiration biopsy, a general anesthetic is recommended to facilitate restraint of the patient and to minimize the risk of stress and trauma to the patient. Infiltration of the subcutis and periosteum with a local anesthetic is also used to provide local analgesia. The anesthetized patient is placed in lateral recumbency, and the hair overlaying the greater trochanter is shaved. The skin is then surgically prepared, and a small incision is made to facilitate passage of the biopsy needle through the skin. The needle is advanced until it reaches the intertrochanteric fossa and is then seated and advanced through the cortical bone and into the marrow space by rotating it in a clockwise-counterclockwise manner.

Once seated within the marrow space, the stylet is removed, and a syringe is attached to the needle hub. Negative pressure is applied until marrow appears just beyond the hub of the needle. Excessive negative pressure and excessive sampling should be avoided to prevent peripheral blood contamination, because marrow samples that are diluted with peripheral blood are difficult to interpret. After collection, the marrow sample is immediately placed in EDTA and/or slides are made before clotting of the sample occurs. To prevent clotting of the sample, it may be advantageous to place two to three drops of 10% EDTA solution in the syringe prior to aspiration.

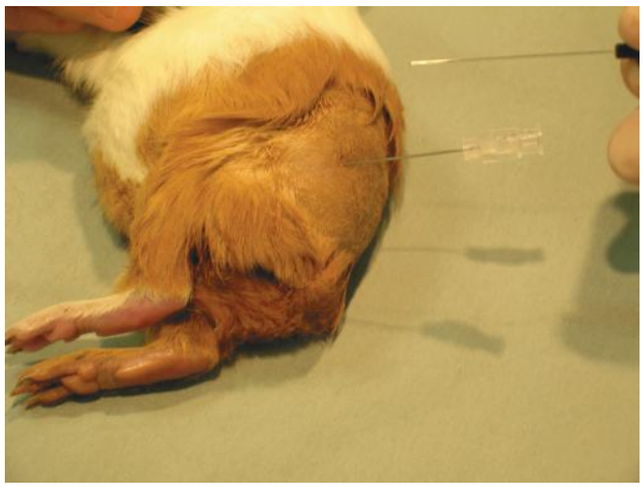


Fig. 1.24. This image shows the stylet removed from the spinal needle that was placed into the proximal femur of a guinea pig (*Cavia porcellus*) for a bone marrow aspiration biopsy.

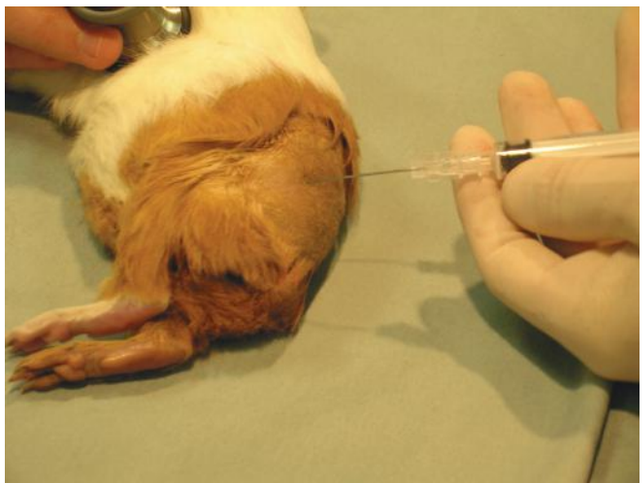


Fig. 1.25. Aspiration of the marrow sample into the lumen of the spinal needle that was placed into the proximal femur of a guinea pig (*Cavia porcellus*) for a bone marrow aspiration biopsy.

Bone marrow films are prepared by placing a drop of marrow on a glass slide, gently placing a spreader slide directly on top with a little or no manual pressure, and then pulling the two glass slides apart after the drop has been allowed to spread. Air-dried slides are stained with a Romanowsky stain.

Core biopsy samples may be obtained using a pediatric bone marrow biopsy needle. The same techniques described for bone marrow aspiration are used for core biopsy collection regarding patient preparation and introduction of the bone marrow needle into the marrow space. Once the marrow space has been entered, a core sample is obtained by advancing the biopsy needle through the marrow space to the opposite cortex after the stylet has been removed. A slight change in the direction of the needle tip movement is made once the end of the needle has reached the opposite cortex to "clip off" the marrow sample inside the needle. No aspiration is performed with this procedure; instead, the needle is withdrawn without replacing the stylet. After removing the needle from the marrow space, the stylet may be used to push the marrow sample out of the lumen of the needle. The core sample may then be rolled across the surface of a glass slide for cytological evaluation before placing it into formalin fixation for histopathology. Romanowsky-stained bone marrow films are evaluated for cellularity and the maturation of the myeloid and erythroid cells. Examination of other cells, such as macrophages, plasma cells, lymphocytes, megakaryocytes, osteoblasts, and osteoclasts, is also part of bone marrow evaluations in Chapter 4.

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